



2014 Joint Annual Meeting of ASAS-ADSA-CSAS  
on July 20-24, 2014, in Kansas City / USA

## EAAP Equine symposium: Genomic research in horses in Europe

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### EAAP - Horse Commission Symposium Genomics and horse production: from science to practice

August 30<sup>th</sup>, 2012 Bratislava, Slovakia  
8.30-18.00



## Scope of this talk:

review & update  
of the high-quality  
scientific work  
in the field of equine  
genomics in Europe



### Session 45. Genomics and horse production: from science to practice

Date: 30 August 2012; 08:30 - 12:30 hours  
Chairperson: Mickelson and Von Velsen-Zerweck

Theatre	Session 45 no.
Genomic signatures of selection in the horse <i>Mickelson, J.R., Petersen, J.L., McCue, M.E. and Valberg, S.J.</i>	1
A mutation in a novel transcription factor affects the pattern of locomotion in horses <i>Andersson, L.S., Schwachow, D., Rubin, C., Arnason, T., Petersen, J.L., McCue, M.E., Mickelson, J.R., Cothran, G., Mikko, S., Lindgren, G. and Andersson, L.</i>	2
Genome-wide association mapping and genomic breeding values for warmblood horses <i>Distl, O., Metzger, J., Schrimpf, R., Philipp, U. and Hilla, D.</i>	3
First results on genomic selection in French show-jumping horses <i>Ricard, A., Danvy, S. and Legarra, A.</i>	4
The myostatin sequence variant g.66493737T>C detects evolution and domestication in horses <i>Dierks, C., Mömke, S., Philipp, U., Lopes, M.S. and Distl, O.</i>	5
Informative genomic regions for insect bite hypersensitivity in Shetland ponies in the Netherlands <i>Schurink, A., Ducro, B.J., Bastiaansen, J.W.M., Frankena, K. and Van Arendonk, J.A.M.</i>	6
Alternative splicing of the elastin gene in horses affected with chronic progressive lymphedema <i>De Keyser, K., Schroyen, M., Oosterlinck, M., Raes, E., Stinckens, A., Janssens, S. and Buys, N.</i>	7
Genetic variation in horse breeds derived from whole genome SNP data <i>Mickelson, J.R., Petersen, J.L. and McCue, M.E.</i>	8
Genomic research in horses: the view of practitioners <i>Von Velsen-Zerweck, A. and Burger, D.</i>	9
Poster	Session 45 no.
Genomic selection in the Swiss Franches-Montagnes horse breed <i>Signer-Hasler, H., Flury, C., Hoase, B., Burger, D., Stricker, C., Simianez, H., Leeb, T. and Rieder, S.</i>	10

## Background

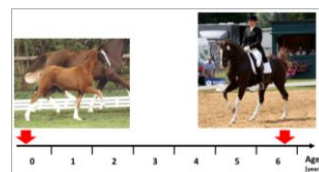
- long tradition of horse breeding in Europe
- multiple breeds and studbooks
  - across and within countries
  - structural heterogeneity
  - intense exchange of genetic material (internationalization)
- breeding programs
  - routine assessments of breeding stock (foals, mares, stallions)
  - highly developed performance testing systems
  - genetic evaluations for conformation and performance traits

**STRONG GENETIC RESEARCH  
IN HORSES IN EUROPE**



## Challenges

- spectrum of traits
  - late availability of phenotype information  
e.g. sport performance
  - limited access to reliable indicator traits  
e.g. stallion performance tests
  - lacking information on important phenotypes  
e.g. health
- approaches to speed up the genetic progress
  - shortening the long generation interval  
→ earlier access to reliable predictions of genetic merit
  - increase of selection intensity for important traits  
→ particularly for the challenging breeding goal traits  
(low  $h^2$ , difficult and limited access to phenotypes)



$$\Delta G = (i * r * \sigma_a) / L$$

with  $\Delta G$  = genetic change,  $\sigma_a$  = genetic variability of the trait,  $i$  = selection intensity,  $r$  = accuracy of selection,  $L$  = generation interval (Falconer 1989)



**high potential of using genomic tools in horses**



## Research framework in Europe

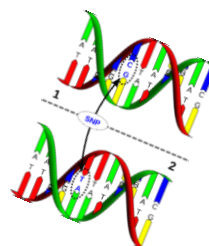
- multi-center molecular genetic / genomic research in equids
- relevant role in the worldwide genome research network
  - internationally recognized scientists and working groups
  - linking of expertise
  - efficient use of resources
- collaboration of science and industry as crucial factor
  - strong equine sector benefitting equine (genomic) research
  - optimum use of genomic tools
  - strategies for studying hereditary conditions

**STRONG GENOMIC RESEARCH  
IN HORSES IN EUROPE**



## Areas of genomic research

- analyses of genome-wide genetic markers (BeadChip analyses)
- Sanger sequencing, Next Generation Sequencing (NGS)
- horse genome assembly (EquCab 3.0)
- population genomics
- genome wide association studies (GWAS)
- mutation analyses



## Horse genome sequence

### ■ 'The Horse Genome Project'

- international consortium:  
≈100 researchers from > 20 countries
- start in 1995, sequence publicly available in  
January 2007: **EquCab1.0**

### ■ EquCab2.0

- Whole Genome Shotgun (WGS) assembly at 6.8X
- sequencing of individuals from multiple breeds  
→ SNP library, 50k SNP chip

### ■ EquCab3.0

- high-throughput Illumina data (42X)
- leverage of long and short read data (NGS)  
for new assembly



Basis of reference genomes:  
'Twilight' (top photo: NINGRI),  
halfbrother 'Bravo' and 'Hrafnhetta'



**SUCCESS OF  
COLLABORATIVE  
GENOMIC RESEARCH**

Wade et al. (2009). Science 326, 865-867. Kalbfleisch et al. (2014). PAG XXII Conference, Jan 10-15, 2014, W279.  
HGP website at <http://www.broadinstitute.org/mammals/horse>; UCSC Genome Bioinformatics at <http://genome.ucsc.edu/>

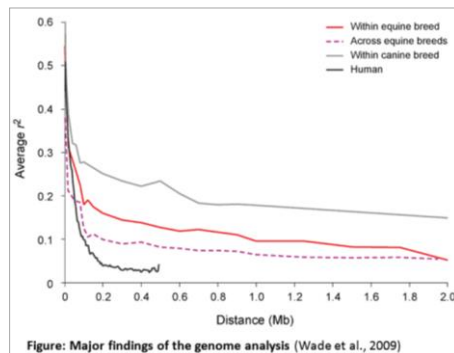
JAM 2014, Equine genomic research (STOCK et al.)

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## Genome sequence → genomic tools (I)

### ■ key facts for further research

- linkage disequilibrium (LD):  
moderate within breeds  
 $LD_{human} < LD_{horse} < LD_{dog}$



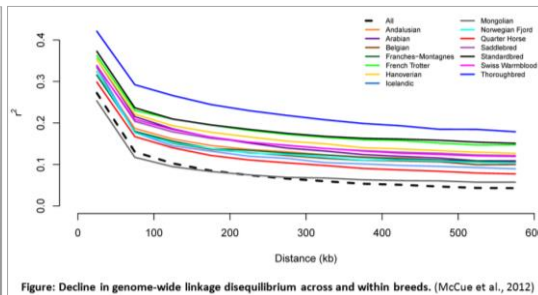
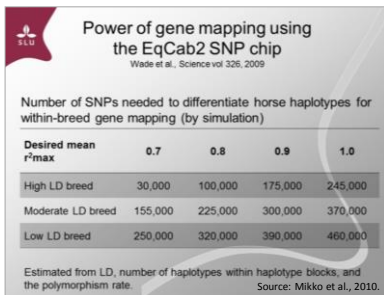
Wade et al. (2009). Science 326, 865-867. Mikko et al. (2010). WCGALP, Aug 1-8, 2010, No.898. McCue et al. (2012). PLoS Genet 8, e1002451.

JAM 2014, Equine genomic research (STOCK et al.)

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## Genome sequence → genomic tools (II)

- key facts for further research
  - linkage disequilibrium (LD): moderate within breeds  
 $LD_{human} < LD_{horse} < LD_{dog}$
  - impact of domestication  
 differences between breeds / breed groups



Wade et al. (2009). Science 326, 865-867. Mikko et al. (2010). WCGALP, Aug 1-8, 2010, No.898. McCue et al. (2012). PLoS Genet 8, e1002451.

JAM 2014, Equine genomic research (STOCK et al.)

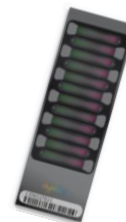
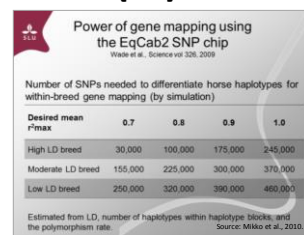
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## Genome sequence → genomic tools (III)

- key facts for further research
  - linkage disequilibrium (LD): moderate within breeds  
 $LD_{human} < LD_{horse} < LD_{dog}$
  - impact of domestication  
 differences between breeds / breed groups

- whole-genome SNP chips
  - Illumina 50k, 70k
  - Affimetrix HD (by the end of 2014)

- implications:
  - facilitated shift of research focus from monogenic to multi-/polygenic traits
  - perspective for routine applications in horse breeding



Wade et al. (2009). Science 326, 865-867. Mikko et al. (2010). WCGALP, Aug 1-8, 2010, No.898. McCue et al. (2012). PLoS Genet 8, e1002451.

JAM 2014, Equine genomic research (STOCK et al.)

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## Target traits

### ■ conformation and functionality

- body size and shape
- conformational defects

### ■ health and disease

- skeleton and locomotor system
- neurological function
- immune system
- metabolism

### ■ performance

- breeding
- racing
- riding



Photo: <http://www.wbfsh.org>

**BREEDING GOAL TRAITS  
IN THE FOCUS OF  
EQUINE GENOMIC RESEARCH**



## Conformation and functionality

### Body size

- model trait for studying genetic influences on quantitative traits (within and across species)
- importance across breeds of horses
  - distinct types of equine skeletal morphology
  - specific body size and shape variation patterns within and across breeds
- increase of knowledge on genetic size regulation
  - particularly fast since 2012



## Conformation and functionality

### Body size: WH (I)

- 1,077 Franches-Montages horses  
212 stallions, 865 broodmares
- withers height of 145-165cm;  
deregressed EBV for withers height
- Illumina EquineSNP50® BeadChip  
(54,602 → 38,124 SNPs)
- QTL regions on ECA3 (*LCORL/NCAPG*)  
and ECA9 (*ZFAT*) together  
explaining 18.2% of dEBV variance

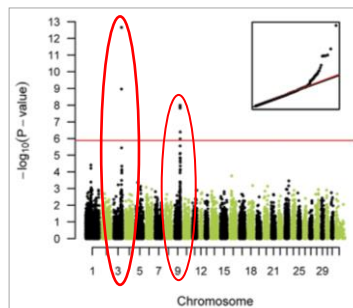


Figure: Manhattan plot for height at withers based on dEBV. (Signer-Hasler et al., 2012)

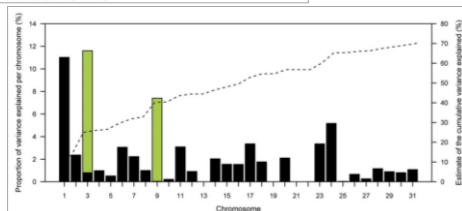


Figure: Estimates of height at withers dEBV variance explained by SNPs on 31 autosomes (black bars) and the two identified QTL (green bars). (Signer-Hasler et al., 2012)

OPEN ACCESS [freely available online](#) **plus one**

### A Genome-Wide Association Study Reveals Loci Influencing Height and Other Conformation Traits in Horses

Heidi Signer-Hasler<sup>1</sup>, Christine Flury<sup>1</sup>, Bianca Haase<sup>2,3</sup>, Dominik Burger<sup>4</sup>, Henner Simianer<sup>5</sup>, Tessa Leeb<sup>6</sup>, Stefan Riederer<sup>7\*</sup>

<sup>1</sup> School of Agricultural, Forest and Food Sciences, Bern University of Applied Sciences, Dübendorf, Switzerland; <sup>2</sup> Institute of Genetics, Veterinary Faculty, University of Bern, Switzerland; <sup>3</sup> Faculty of Veterinary Science, University of Sydney, New South Wales, Australia; <sup>4</sup> Equine Research Laboratory, University of Guelph, Ontario, Canada; <sup>5</sup> Institute of Animal Breeding and Biotechnology, University of Applied Sciences, Hohenheim, Germany; <sup>6</sup> Institute of Animal Breeding and Biotechnology, University of Applied Sciences, Hohenheim, Germany; <sup>7</sup> Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

PLoS ONE 7(5): e37282. doi:10.1371/journal.pone.0037282

Received January 5, 2012; Accepted April 13, 2012; Published May 16, 2012

JAM 2014, Equine genomic research (STOCK et al.)

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## Conformation and functionality

### Body size: WH (II)

- 782 young German WB stallions  
Hol. (N=333), Old. (N=213), Han. (N=192), Trak. (N=44)
- mean withers height of 166.1 ± 2.34cm,  
age of 2.5 years
- Illumina EquineSNP50® BeadChip  
(54,602 → 42,642 SNPs)
- single QTL region on ECA3 (*LCORL/NCAPG*)  
explaining 18% of phenotypic variance

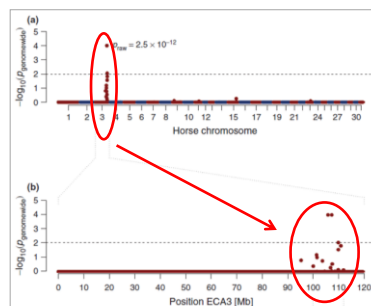


Figure: Manhattan plot for height at withers based on measurements. (Tetens et al., 2013)

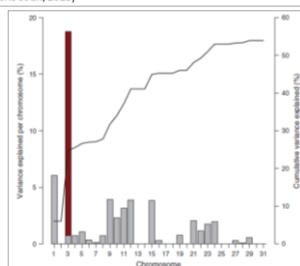


Figure: Estimates of phenotypic height at withers variance explained by SNPs on 31 autosomes (grey bars) and the identified QTL (red bar). (Tetens et al., 2013)

Faculty of Agricultural and Nutritional Sciences **CIAU**

### A genome wide association study for withers height in German Warmblood Horses

Christa Kühn<sup>1</sup>, Jens Tetens<sup>1</sup>, Philipp Widmann<sup>2</sup>, Georg Thaller<sup>1</sup>

<sup>1</sup> Institute of Animal Breeding and Biotechnology, Christian-Albrechts-University Kiel, Germany; <sup>2</sup> Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

33rd ISAG Conference, Cairns, Australia, July 15-20th, 2012.

**ANIMAL GENETICS** Immunogenetics, Molecular Genetics and Functional Genomics

SHORT COMMUNICATION doi:10.1111/age.12091

### A genome-wide association study indicates *LCORL/NCAPG* as a candidate locus for withers height in German Warmblood horses

J. Tetens<sup>1</sup>, P. Widmann<sup>2</sup>, C. Kühn<sup>1</sup> and G. Thaller<sup>1</sup>

JAM 2014, Equine genomic research (STOCK et al.)

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## Body size: WH (III)

- GWAS: 214 Hanoverian WB horses; 1,851 horses from **42 breeds** (average withers height 83-170 cm)
- Illumina EquineSNP50® BeadChip (54,602 → 44,496 SNPs)
- expression analysis: 47 horses from 5 breeds
- significant association of relative LCORL expression levels with SNP genotype (T>C mutation) and body size ('pony mutation' / ≤148cm)

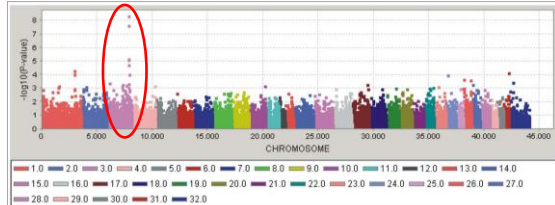


Figure: Genome-wide association analysis shows a highly significant peak on equine chromosome (ECA) 3 in Hanoverian. (Metzger et al., 2013)

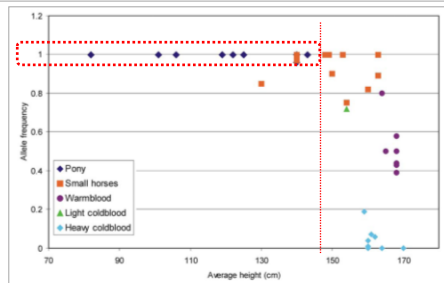


Figure: Averaged height at the withers plotted against the frequency of the allele T. (Metzger et al., 2013)

Expression Levels of *LCORL* Are Associated with Body Size in Horses  
Julia Metzger, Rahel Schrimpf, Ute Philipp, Ottmar Distl  
Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Hannover, Germany  
PLoS ONE 8(2): e56897. doi:10.1371/journal.pone.0056897  
Received August 26, 2012; Accepted January 10, 2013; Published February 13, 2013

## Body size (cont.)

- model trait for studying genetic influences on quantitative traits (within and across species)

- importance across breeds of horses

- distinct types of equine skeletal morphology
- specific body size and shape variation patterns within and across breeds

- increase of knowledge on genetic size regulation:

**LCORL** (ligand-dependent nuclear receptor compressor-like protein)

- consistent GWAS results in horses  
Franches-Montagne horses (Signer-Hasler et al., 2012)  
German warmblood horses (Kühn et al., 2012 / Tetens et al., 2013; Metzger et al., 2013)  
Arabs (Ricard et al., 2013)  
multiple breeds and breed types (Metzger et al., 2013)
- relevant size regulator across species  
humans (Lango Allen et al., 2010), cattle (Pryce et al., 2011)





## Skeleton and locomotor system

- major role of locomotor health for (long-term) usability of horses
- some conditions with significant prevalences across breeds, e.g. juvenile osteochondral conditions (JOCC) (Denoix et al., 2013)

### ■ osteochondrosis (OC)

- focal failure of enchondral ossification
- considerable impact on the market value of horses
- extensively studied worldwide for a long time (clinics, populations genetics, molecular genetics)
- multifactorial
- accumulating knowledge
  - faster now with the help of genomics



Osteochondritis dissecans (OCD) in the fetlock joint

Denoix et al. (2013). Vet J 197, 29-35.

JAM 2014, Equine genomic research (STOCK et al.)

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## Skeleton and locomotor system: OC (I)

- 1,162 young German WB stallions
- radiographic protocols (binary OC/OCD)
- Illumina EquineSNP50® BeadChip (54,602 → 44,410 SNPs; 916 stallions)
- distinct results for hock and fetlock / toe, indications of major QTL on ECA20 (OCD fetlock / toe)

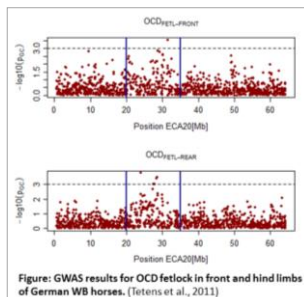


Figure: GWAS results for OCD fetlock in front and hind limbs of German WB horses. (Tetens et al., 2011)

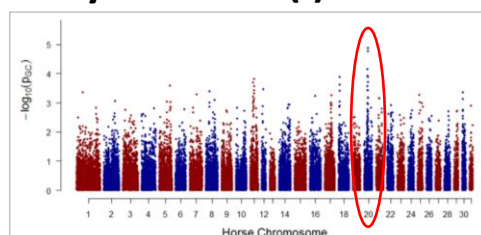


Figure: GWAS results for OCD fetlock in German WB horses. (Tetens et al., 2011)

impact of trait definition!  
importance of specific & reliable  
phenotype information!

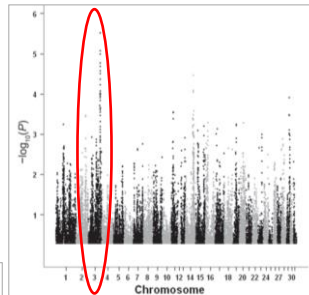
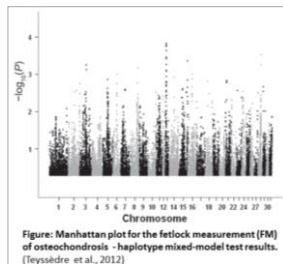
Tetens et al. (2011). EAAP Annual Meeting, Starvanger, Norway.

JAM 2014, Equine genomic research (STOCK et al.)

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## Skeleton and locomotor system: OC (II)

- 583 French trotters
- radiographic protocols (scores, binary)
- Illumina EquineSNP50® BeadChip (54,602 → 41,249 SNPs)
- no evidence for major gene, no common SNP associations for hock OC (ECA3) and fetlock OC



impact of trait definition!  
importance of specific & reliable  
phenotype information!

Teyssèdre et al. (2012). J Anim Sci 90, 45-53.

JAM 2014, Equine genomic research (STOCK et al.)

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## Skeleton and locomotor system: OC (III)

- QTL for major OC/OCD conditions reported on >20 chromosomes
- some chromosomes with >1 QTL
- confirmed and new QTL in recent GWAS (50k / 70k SNP chips)

Table: Genomic regions associated with osteochondrosis (OC) as shown by GWAS in Hanoverian Warmblood (HW), French Trotter (FT), Norwegian Trotter (NT) and English Thoroughbred (TB) horses. (Distl, 2013)

ECA	Breed	Trait	Position in Mb
1	HW	OC, OCD, OC-F, OCD-F, OC-H, OCD-H	43-45
2	HW	OC-F, OCD-F	104
3	HW	OC, OCD	64-65
3	TB	OCD	88
3	FT	OC-H	102-107
4	HW	OC-F, OCD-F, OC-H, OCD-H	41
5	NT	OCD-H	42, 77
10	NT	OCD-H	80
13	FT	OC-F	9-12
14	FT	OC-H	67-76
15	FT	OC-F	87-89
16	HW	OC-F, OCD-F	81
18	HW	OC, OCD	36
26	HW	OC-F, OCD-F	27
27	NT	OCD-H	38
28	NT	OCD-H	42
30	HW	OC-F, OCD-F	87

ECA = Equus caballus autosome; OC (OCD) = osteochondrosis (osteochondrosis dissecans) in fetlock and/or hock joints; OC-F (OCD-F) = osteochondrosis (osteochondrosis dissecans) in fetlock joints; OC-H (OCD-H) = osteochondrosis (osteochondrosis dissecans) in hock joints.  
References: Corbin et al., 2012; Lampe et al., 2009; Lykijien et al., 2010; Teyssèdre et al., 2012.

Table: Quantitative trait loci (QTL) for osteochondrosis (OC) in Hanoverian Warmblood (HW) and South German Coldblood (SGC) horses. (Distl, 2013)

ECA	Breed	Phenotypic trait	Position in Mb
1	SGC	OC-F, OCD-F	161-182
2	HW	OC, OCD, OC-F, OCD-F, OC-H	19-37
3	HW	OCD, OCD-F	11-16
4	HW	OC, OC-F, OC-H	3-13
	HW	OC, OC-F	27-29
	HW	OC, OC-F	56-60
	SGC	OCD-F	50-66
5	HW	OCD-H	44-53
	HW	OC-F, OCD-F	76-93
	SGC	OC, OC-F	53-57
13	SGC	OCD-F	4-7
15	HW	OC-H	63-64
15	SGC	OC, OC-H	18-39
16	HW	OC, OCD, OC-F	6-24
	HW	OCD-H	33-45
	SGC	OC-F	23-28
17	SGC	OC, OC-F	41-42
18	HW	OC, OCD-H	74-82
18	SGC	OC-F, OCD-F, OC-H	37-52
19	HW	OCD	0-1
21	HW	OC-H, OCD-H	5-17
22	SGC	OC, OCD-F	14-15, 31-48
23	SGC	OC, OC-F	37
25	SGC	OC, OC-F, OCD-F	0-1
27	SGC	OC-F	12-15
28	SGC	OC, OC-F	9-11
31	SGC	OC-H	21-22

ECA = Equus caballus autosome; OC (OCD) = osteochondrosis (osteochondrosis dissecans) in fetlock and/or hock joints; OC-F (OCD-F) = osteochondrosis (osteochondrosis dissecans) in fetlock joints; OC-H (OCD-H) = osteochondrosis (osteochondrosis dissecans) in hock joints.  
References: Dierks et al., 2007; Dierks et al., 2010a,b; Felicetti et al., 2008; Lampe et al., 2009; Wittwer et al., 2007; Wittwer et al., 2008.

impact of trait definition & study design!

Distl (2013). Vet J 197, 13-18.

JAM 2014, Equine genomic research (STOCK et al.)

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## Skeleton and locomotor system (cont.)

- major role of locomotor health for (long-term) usability of horses
- some conditions with significant prevalences across breeds, e.g. juvenile osteochondral conditions (JOCC) (Denoix et al., 2013)

### ■ osteochondrosis (OC)

- focal failure of enchondral ossification
- considerable impact on the market value of horses
- extensively studied worldwide for a long time (clinics, populations genetics, molecular genetics)
- multifactorial
- accumulating knowledge: set of distinct conditions to be considered as different multigenic traits
  - OC and OCD in fetlock, hock and stifle joints
  - fetlock: dorsodistal osteochondral fragments (DOFs)
  - plantar osteochondral fragments (POFs) of hind limbs



Osteochondral dissecans (OCD) in the fetlock joint

Denoix et al. (2013). Vet J 197, 29-35.

## Immune system

- severe impact of immunologic dysfunction across species
  - defense mechanisms (infections)
  - autoimmune disorders
  - allergic reactions

## Immune system: ERU

### ■ severe impact of immunologic dysfunction across species

- defense mechanisms (infections)
- **autoimmune disorders**
- allergic reactions

### ■ equine recurrent uveitis (ERU)

- long-known eye condition of significant prevalence in multiple breeds
- most common cause of blindness in horses
- link between *Leptospira spp.* and chronic recurrence of intraocular inflammation
- current knowledge: most likely autoimmune uveitis



Photo: <http://www.davidwilliams.org.uk>

- 144 German WB horses
- ERU score 0-3, binary  
 $N_{ERU0}=65$ ,  $N_{ERU1-3}=25+30+24$
- Illumina EquineSNP50® BeadChip (54,602 → 37,040 SNPs)
- significantly associated SNPs: ECA20 (binary; IL-17A, IL-17F), ECA18 (score; CRYGA-CRYGF)

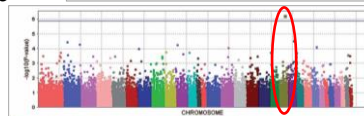


Figure 1 Case-control genome-wide association study for equine recurrent uveitis (ERU) in German WB horses. (Kulbrock et al., 2013)

Kulbrock et al. (2013). PLoS ONE 8, e71619.

## Immune system: IBH

### ■ severe impact of dysfunction across species

- defense mechanisms (infections)
- autoimmune disorders
- **allergic reactions**

### ■ equine recurrent uveitis (ERU)

### ■ equine insect bite hypersensitivity (IBH)

- long-known allergic skin condition of significant prevalence in multiple breeds
- link between *Culicoides spp.* and chronic recurrence of skin inflammation
- current knowledge: role of multiple genes related to immune function
  - ELA (equine leucocyte antigen) class II (Anderson et al., 2012)
  - IGHE (immunoglobulin heavy constant  $\epsilon$ ) (Wagner et al., 2001)



Photo: <http://www.vetnext.com>

- 187 Icelandic horses (SWE), 186 Exmoor ponies (UK)  
94+80 cases, 93+106 controls
- IBH status (binary)
- Illumina EquineSNP50® BeadChip (54,602 → 44,200 SNPs; only IH); microsatellites, sequence data
- MHC class II region: association of same variants and of regional homozygosity with IBH susceptibility

Andersson et al. (2012). Immunogenetics 64, 201-208. Wagner et al. (2001). Vet Immunol Immunopath 92, 193-202.

## Neurological function

- highly complex interplay of peripheral and central nervous function
  - some very specific functional defects
  - understanding of regular function (across species)  
often driven by studies of dysfunction (in target and model species)

## Neurological function: RLN (I)

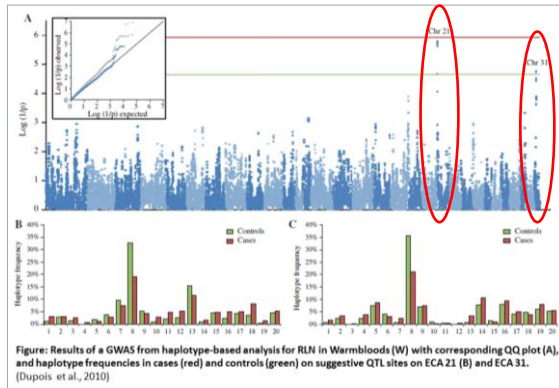
- highly complex interplay of peripheral and central nervous function
  - some **very specific functional defects**
  - understanding of regular function (across species)  
often driven by studies of dysfunction (in target and model species)
- **recurrent laryngeal neuropathy (RLN)**
  - obstructive condition of the upper respiratory tract with possible impact on performance
  - link between body size and 'roaring'
  - current knowledge:  
specific mononeuropathy with familial disposition



Photo: S. Brard

## Neurological function: RLN (II)

- 531 horses  
234 cases + 228 controls (incl. 196+188 WB),  
69 parents
- endoscopic score for RLN  
17 veterinary clinics in FRA, BEL, D, CH
- Illumina EquineSNP50® BeadChip  
(54,602 → 47,046 SNPs)
- no evidence of major gene, but  
suggestive SNPs on ECA21 and ECA31  
for WB (enrichment of 'protective'  
haplotype in controls)



importance of specific & reliable  
phenotype information!

Dupuis et al. (2011). Mamm Genome 22, 613-620.

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## Neurological function: Locomotion (I)

- highly complex interplay of peripheral and central neural function
  - some very specific functional defects
  - understanding of regular function** (across species)  
often driven by studies of **specific function** (in target and model species)
- recurrent laryngeal neuropathy (RLN)
- gaitedness and high-speed trotting**
  - breed-specific ability to perform alternate gaits, i.e. surplus to basic gaits (walk, trot, canter):  
pace, ambling gaits (regular rhythm, lateral, diagonal ambling)
  - breed-specific ability to sustain trot at high speed, i.e. delay transition into canter
  - related to general control / coordination of limb movement



Photo: Andersson, 2012

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## Neurological function: Locomotion (II)

- 70 Icelandic horses  
40 with pace (5-gaited), 30 without pace (4-gaited)
- 2 Icelandic horses  
1 with pace (5-gaited, AA), 1 without pace (4-gaited, CC)
- capability of pace (Y/N, competition records)
- Illumina EquineSNP50® BeadChip  
(54,602 SNPs), sequence data;  
mouse model (wild-type and Dmrt3-null mice)
- GWAS: strong association signal on ECA23
- mutation detection: nonsense mutation C/A  
(transcription factor DMRT3)  
→ premature stop codon  
(truncated protein, 174 amino acids less)
- DMRT3 gene expression: subset of inhibitory  
neurons in spinal cord (crossing dorsal midline,  
connecting to motor neurons)

Table: Distribution of SNP genotypes.  
(Andersson et al., 2012)

	CC/CT	TT
pace	1	39
not pace	21	9

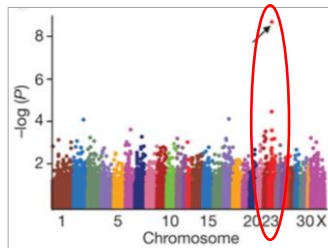


Figure: GWAS results for the ability to pace with genome-wide significant SNP on ECA23. (Andersson et al., 2012)

Andersson et al. (2012). Nature 488, 642-646. Andersson (2012). EAAP Annual Meeting, Bratislava, Slovakia.

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## Neurological function: Locomotion (III)

- ...
- 352 Icelandic horses,  
176 gaited horses (6 breeds),  
218 non-gaited horses (8 breeds),  
414 horses bred for harness racing
- high frequencies of DMRT3 mutation  
in gaited and harness racing horses
- DMRT3 mutation:  
permissive for performing alternate gaits,  
i.e. pace or four-beat ambling gaits, and  
high-speed trot ('gait keeper mutation')

Table: Distribution of the DMRT3 mutation in the Icelandic horse.  
(Andersson et al., 2012)

Phenotype	CC	CA	AA	Total
Five-gaited	0	1	65	66
Four-gaited	2	83	39	124
Total	3	105	149	257

$p < 2.4 \times 10^{-14}$



Table: Allele frequency of the DMRT3 nonsense mutation among horse populations. (Andersson et al., 2012)

Breed	n	p(A)
Icelandic horses*		
Four-gaited	124	0.65
Five-gaited	66	0.99
Random sample	162	0.89
Other gaited horses		
Kentucky mountain saddle horse	22	0.95
Missouri fox trotter	40	1.00
Paso fino	45	1.00
Peruvian paso	19	1.00
Rocky mountain horse	17	1.00
Tennessee walking horse	33	0.98
Non-gaited horses		
Arabian horse	18	0.00
Gotland pony	28	0.00
North-Swedish draft horse	31	0.00
Przewalski's horse	6	0.00
Shetland pony	20	0.00
Swedish ardenneres	22	0.00
Swedish warmblood	64	0.00
Thoroughbred	29	0.00
Horses bred for harness racing		
Standardbred, trotter (Sweden)	270	0.97
Standardbred, trotter (USA)	57	1.00
Standardbred, pacer (USA)	40	1.00
French trotter (France)	47	0.77

Andersson et al. (2012). Nature 488, 642-646. Andersson (2012). EAAP Annual Meeting, Bratislava, Slovakia.

JAM 2014, Equine genomic research (STOCK et al.)

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## Performance

# Racing and riding



Photo: Ricard et al., 2012



Photo: <http://www.wbfsn.org>



- performance criteria
  - mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
  - some characteristics of relevance in either discipline
- 'total picture': locomotion pattern, physiological characteristics, behavior, ...

## Performance

# Racing and riding: racing (I)



- performance criteria
  - mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
  - some characteristics of relevance in either discipline
- 'total picture': locomotion pattern, physiological characteristics, behavior, ...

- 659 French **trotters**
- trotting race records (probability of qualification test, proportion of disqualified races, earnings)
- *DMRT3* genotyping; Illumina EquineSNP50® BeadChip
- *DMRT3*: no fixation of AA genotype, association of CC genotype with weaker performance (QT probability ↓, disqualification rate ↑, earnings ↓), intermediate results for CA genotype (QT probability ↓, earning of 5-years-olds ↑)
- GWAS: some stronger associated SNPs

Ricard (submitted).





## Performance

### Racing and riding: racing (II)

- performance criteria
  - mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
  - some characteristics of relevance in either discipline
- 'total picture': locomotion pattern, physiological characteristics, behavior, ...

- 597 **Arabian endurance horses**
- endurance race records (speed, distance, rates of finished races)
- Illumina EquineSNP70® BeadChip (74k → 56,200 SNP)
- no significantly associated SNPs for speed, but 2 SNPs on ECA6 and 1 SNP on ECA7 for distance and finishing rates



Ricard et al., (2013). 10th Internati. Equine Gene Mapping Workshop, Azores, Portugal, July 10-13, 2013.

## Performance

### Racing and riding: riding (I)

- performance criteria
  - mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
  - some characteristics of relevance in either discipline
- 'total picture': locomotion pattern, physiological characteristics, behavior, ...

- 115 German WB stallions
- breeding values for jumping  
basis: scores for **free jumping**; BV range 56 - 171, reliability 0.87
- Illumina EquineSNP50® BeadChip (54,602 → 43,441 SNP)
- QTL on ECA1, 8, 9 and 26, further putative QTL on ECA 1, 3, 11, 17,217
- genotype-based breeding values:  
sum of additive and dominance effects of 6 SNPs explaining 54% of variance of conventional BV



Schröder et al. (2011). Anim Genet 43, 392-400.

## Racing and riding: riding (II)

### ■ performance criteria

- mostly use-specific criteria in different horse breeds (breed formation, generations of human selection)
- some characteristics of relevance in either discipline

### ■ 'total picture':

locomotion pattern, physiological characteristics, behavior, ...

- 908+102 **show-jumping** horses, 289 **3-day-event** horses (French sport horse, Anglo-Arabian, other European sport horses)
- de-regressed breeding values for **competition** performance (basis: ranking and points, previously money earned)
- Illumina EquineSNP50® BeadChip (54,602 → 44,424 SNP for GWAS, 44,444 for GS)
- GWAS: no evidence of major gene, but for show-jumping suggestive QTL on ECA1 (Ryanodine Receptor 2)
- GS show-jumping (908 horses): only slight improvement of prediction accuracy ( $r^2_{\text{convGE}}=0.36$ ,  $r^2_{\text{GS}}=0.39$ )



Ricard et al. (2012). EAAP Annual Meeting, Bratislava, Slovakia. Ricard et al. (2013). J Anim Sci 91, 1076-1085. Brad & Ricard (submitted).

## Target traits & genomic approaches

### ■ increasing complexity of phenotypes

implying increasing challenges (also) in genomic research

- conformation and functionality
- health and disease
- performance

### ■ maximum use of the prospects of genomics

- high standards for phenotyping
- efficient use of resources (samples, data, genomic tools, knowledge)



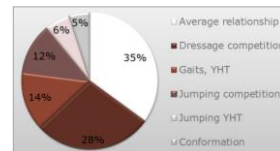
### ■ recent development of genomic research in horses in Europe

- several initiatives with joint engagement of science and industry
- national and international consortium partners

## Recent developments

# Danish project on GS

- focus on **performance and health**
- national consortium  
(open for international collaboration)
- selection of  $\approx 500$  horses for genotyping
  - DNA availability, low average relationship
  - EBV of as high as possible accuracy  
competition, young horse performance, conformation (health)
- strategy
  - moderate + high density genotyping  
majority of horses 70k, few selected horses 670k
  - inclusion of genomic information  
in regular breeding evaluations  
phenotypes: young horse and competition results  
single step procedure (challenge: proper scaling)



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## Recent developments

# Horsegene

- **heritable diseases** of horses  
with severe impact on the horse sector
- international consortium:  
BEL, SWE, UK, NL
- strategy
  - samples over several breeds  
samples sizes (cases+controls):  
900+900 for insect bite hypersensitivity (IBH)  
300+200 for chronic progressive lymphedema (CPL)  
600+600 for osteochondrosis (OC)
  - identification of genes and genetic markers  
high-density genotyping,  
pooled genome sequencing (cases/controls),  
sequencing of key ancestors and imputation
  - implementation of genomic selection  
against disease susceptibility in breeding programs



Photo: <http://www.horsegene.eu>

## SME partners

- British Equestrian Federation
- Koninklijk Fries Paarden Stamboek
- Svenska Islandshästförbundet
- Belgisch Warmbloed Paard
- Swedish Horse Board
- Koninklijke Maatschappij Belgisch Trekpaard
- Studfarm De Breemeersen
- Stal Groeneweg B.V.
- Ostra Greda Islandshästgard
- Van Haeringen Laboratoires

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## Future of genomic research

- expansion of collaboration in the equine sector in Europe
  - strengthening of the whole sector
  - clearly less concerns of the horse industry to join efforts in the new field (genomics) than in the traditionally studbook-specific breeding issues

⇒ expected: joint European research activity in equine genomics ↑
- scenarios for international collaboration
  - exchange of genotypes  
genotyped sires used for breeding in several studbooks / countries
  - joint genomic evaluation  
exchange of genotypes, phenotypes and pedigree information



# Thank you!

